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GENOME ANNOUNCEMENT

Draft Genome Sequence of the Volcano-Inhabiting Thermoacidophilic Methanotroph *Methylacidiphilum fumariolicum* Strain SolV

Ahmad F. Khadem,[a] Adam S. Wieczorek,[a] Arjan Pol,[a] Stéphane Vuilleumier,[b] Harry R. Harhangi,[a] Peter F. Dunfield,[c] Marina G. Kalyuzhnaya,[d] J. Colin Murrell,[e] Kees-Jan Francoijs,[f] Henk G. Stunnenberg,[f] Lisa Y. Stein,[g] Alan A. DiSpirito,[h] Jeremy D. Semrau,[i] Aurélie Lajus,[j] Claudine Médigue,[j] Martin G. Klotz,[k] Mike S. M. Jetten,[a] and Huub J. M. Op den Camp[a]

Department of Microbiology, IWWR, Radboud University Nijmegen, Nijmegen, the Netherlands[a]; Université de Strasbourg, UMR 7156 CNRS, Strasbourg, France[b]; Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada[c]; Department of Microbiology, University of Washington, Seattle, Washington, USA[d]; School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, United Kingdom[e]; Department of Molecular Biology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, the Netherlands[f]; Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada[g]; Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa, USA[h]; Department of Civil and Environmental Engineering, The University of Michigan, Ann Arbor, Michigan, USA[i]; Laboratoire d'Analyses Bioinformatiques pour la Génomique et le Métabolisme (LABGeM), Genoscope-IG-CEA, Evry, France[j]; and Department of Biology, University of North Carolina, Charlotte, North Carolina, USA[k]

The draft genome of *Methylacidiphilum fumariolicum* SolV, a thermoacidophilic methanotroph of the phylum *Verrucomicrobia*, is presented. Annotation revealed pathways for one-carbon, nitrogen, and hydrogen catabolism and respiration together with central metabolic pathways. The genome encodes three orthologues of particulate methane monooxygenases. Sequencing of this genome will help in the understanding of methane cycling in volcanic environments.

Isolation (14) and genome sequencing of strain SolV led to the proposal that *Methylacidiphilum fumariolicum* be one of three proposed species within the genus *Methylacidiphilum* (13), together with *M. infernorum* (strain V4) (4) and *M. kamchatkensis* (strain Kam1) (7). All three strains were isolated from acidic volcanic areas and are well adapted to the harsh volcanic environment (13, 14), being able to thrive at very low methane and oxygen concentrations and pH values as low as 1.

The high-quality draft genome of *M. fumariolicum* SolV (109 contigs) was assembled from Illumina and Roche 454 reads using CLCbio (CLCbio, Aarhus, Denmark) and manual adjustments. The draft genome is 2.36 Mbp in size, with a GC content of 40.9%. Auto-annotation was performed based on comparison to public databases using the MicroScope platform (Genoscope, France) (17), which identified 2,283 protein-encoding gene models. For 623 of these, full-length homologs (>80% identity at the protein level) were present in the complete genome of *M. infernorum* V4 (6), with 619 of them organized in synteny in the two strains. Biosynthetic pathways and tRNAs of all 20 amino acids were present together with a single rRNA operon.

Key genes for the ribulose monophosphate pathway and the serine cycle were absent. However, genes encoding the Calvin-Benson-Bassham cycle enzymes were present, supporting physiological studies (9). The genome encodes all three central pathways: the Embden-Meyerhof-Parnas glycolytic pathway, the pentose phosphate pathway, and the tricarboxylic acid cycle. Genes encoding keto-deoxy-gluconate catabolism of the Entner-Doudoroff pathway were absent. Three particulate methane monooxygenase operons (*pmoCAB*) were predicted, while genes encoding soluble methane monooxygenase were not found. The *mxaFI* genes encoding methanol dehydrogenase (2, 3) were absent, but a homologous *xoxFJG* gene cluster and a *pqqABCDEF* operon for the biosynthesis of the cofactor pyrroloquinoline quinone were detected. H_4MPT-linked C_1 transfer genes are not present. The H_4Folate-linked pathway inventory includes *metF*, *folD*, and *ftfL* genes. *mtdA*, *fch*, and *purU* were not found. Genes encoding NAD-linked formate dehydrogenase (*fdsABG*) were identified (12). Should the identified genes encoding acetate kinase and acetyl-coenzyme A synthase prove functional, strain SolV may be able to assimilate C_2 compounds and thus be a facultative methanotroph (15). The presence of a hydrogenase gene cluster points toward possible chemolithotrophic growth or the use of hydrogen to provide reducing equivalents for methane oxidation (5). A complex IV-type heme-copper oxidase gene cluster possibly encodes the terminal cytochrome *c* oxidase.

Strain SolV was able to grow with ammonium, nitrate, or dinitrogen gas as a nitrogen source (8, 14). Coincidentally, genes were predicted for direct ammonium uptake (*amtB*) and assimilation (e.g., glutamine synthase, *glnA*; glutamate synthase, *gltB*; alanine dehydrogenase, *ald*) as well as for urea metabolism. As in most other methanotrophs, however, the urea cycle is incomplete (1). A full complement of genes for dinitrogen fixation, nitrate/nitrite transport, and assimilation was also found. In addition, genes for nitrite reduction (*nirK*) and nitric oxide reduction (*norB norC*) were identified, but the inventory to encode nitrous oxide reduction was missing. A *haoAB* gene cluster encoding hydroxylamine oxidase was identified, suggesting the capability of nitrification and nitrosative stress handling (10, 11, 16).

Nucleotide sequence accession numbers. The nucleotide genome sequence of *M. fumariolicum* SolV has been deposited in the European Nucleotide Archive (ENA) under accession numbers CAHT01000001 to CAHT01000109.

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Address correspondence to Huub J. M. Op den Camp, h.opdencamp@science.ru.nl.

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REFERENCES

1. **Boden R, et al.** 2011. Complete genome sequence of aerobic marine methanotroph *Methylomonas methanica* MC09. J. Bacteriol. **193**:7001–7002.
2. **Chen Y, et al.** 2010. Complete genome sequence of the aerobic facultative methanotroph *Methylocella silvestris* BL2. J. Bacteriol. **192**:3840–3841.
3. **Chistoserdova L, Kalyuzhnaya MG, Lidstrom ME.** 2009. The expanding world of methylotrophic metabolism. Annu. Rev. Microbiol. **63**:477–499.
4. **Dunfield PF, et al.** 2007. Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature **450**:879–882.
5. **Hanczár T, Csáki R, Bodrossy L, Murrell JC, Kovács K.** 2002. Detection and localization of two hydrogenases in *Methylococcus capsulatus* (Bath) and their potential role in methane metabolism. Arch. Microbiol. **177**: 167–172.
6. **Hou S, et al.** 2008. Complete genome sequence of the extremely acidophilic methanotroph isolate V4, *Methylacidiphilum infernorum*, a representative of the bacterial phylum *Verrucomicrobia*. Biol. Direct **3**:26.
7. **Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK.** 2008. Methane oxidation at 55°C and pH 2 by a thermoacidophilic bacterium belonging to the Verrucomicrobia phylum. Proc. Natl. Acad. Sci. U. S. A. **105**:300–304.
8. **Khadem AF, Pol A, Jetten MSM, Op den Camp HJM.** 2010. Nitrogen fixation by the verrucomicrobial methanotroph *Methylacidiphilum fumariolicum* SolV. Microbiology **156**:1052–1059.
9. **Khadem AF, et al.** 2011. Autotrophic methanotrophy in Verrucomicrobia: *Methylacidiphilum fumariolicum* SolV uses the Calvin Benson Bassham cycle for carbon dioxide fixation. J. Bacteriol. **193**:4438–4446.
10. **Nyerges G, Han SK, Stein LY.** 2010. Effects of ammonium and nitrite on growth and competitive fitness of cultivated methanotrophic bacteria. Appl. Environ. Microbiol. **76**:5648–5651.
11. **Nyerges G, Stein LY.** 2009. Ammonia co-metabolism and product inhibition vary considerably among species of methanotrophic bacteria. FEMS Microbiol. Lett. **297**:131–136.
12. **Oh JI, Bowien B.** 1998. Structural analysis of the *fds* operon encoding the NAD+-linked formate dehydrogenase of *Ralstonia eutropha*. J. Biol. Chem. **273**:26349–26360.
13. **Op den Camp HJM, et al.** 2009. Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environ. Microbiol. Rep. **1**:293–306.
14. **Pol A, et al.** 2007. Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature **450**:874–878.
15. **Semrau JD, DiSpirito AA, Vuilleumier S.** 2011. Facultative methanotrophy: false leads, true results, and suggestions for future research. FEMS Microbiol. Lett. **323**:1–12.
16. **Stein LY, Klotz MG.** 2011. Nitrifying and denitrifying pathways of methanotrophic bacteria. Biochem. Soc. Trans. **39**:1826–1831.
17. **Vallenet D, et al.** 2009. MicroScope: a platform for microbial genome annotation and comparative genomics. Database (Oxford) Article ID :bap021. doi:10.1093/database/bap021.